

BACKGROUND

Enceladus, a small, inner moon of Saturn, has lately been of great interest to many astrobiologists. The entire surface of the moon is covered with a layer of ice water and reflects almost 100% of the light that strikes its surface. With a gravity of 0.1 m/s² and only 500 km across, Enceladus seems unlikely to harbor much geological activity; however, the moon is quite active particularly in its South Pole[1]. Observations by the Composite Infrared Spectrometer instrument on the spacecraft Cassini revealed unusually high thermal readings along the "tiger stripe" region in the South. The combination of the geological activity and high temperature readings have led to speculations of a subsurface liquid water aquifer. In July 2005, the Cassini witnessed a plume erupting from the southern pole of Enceladus. Instruments on board the spacecraft were able to take several measurements to analyze the ejected particles in the plume. Results showed that the plume contained water vapor, simple organic compounds such as methane, and some level of N₂ or CO or both[2]. The methane detected in the plume may have biological origins.

PURPOSE AND HYPOTHESIS

Purpose: These experiments were designed to determine what level of ammonium chloride would allow for methane production by *Methanothermobacter wolfeii* and *Methanococcus maripaludis*.

Hypothesis: Both methanogens should grow at low concentrations (i.e. 1-2%) of ammonium chloride, but *M. maripaludis* should grow at higher concentrations (i.e. 4-6%) since the microorganism is halophilic.

% NH ₄ Cl	% NaCl	Total Salt (%)
0	4	4
1	3	4
2	2	4
3	1	4
4	0	4

Table 1: Varying ammonium chloride concentrations though all tubes had a total salt concentration of 4% to eliminate salt as a variable.

METHODS

- *M. maripaludis* and *M. wolfeii* were both grown in MS medium which already contained the various salt concentrations, as shown in Table 1.
- *M. wolfeii* is a thermophilic microorganism and must be incubated at 55C[3].
- *M. maripaludis* survives at room temperature, which is approximately 25C.
- Both methanogens are anaerobic microorganisms, and must be kept in an environment deprived of oxygen.

RESULTS

EXPERIMENT 1

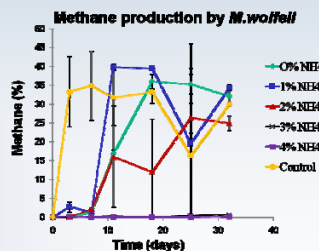


Figure 1: This graph shows that *M. wolfeii* grows at 0%, 1%, and 2% concentrations of ammonium chloride.

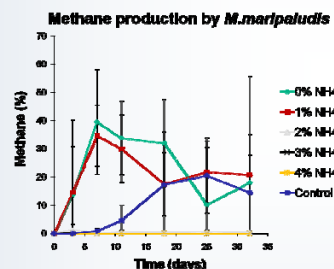


Figure 2: *M. maripaludis* grows well at 0% and 1% ammonium chloride. Due to its halophilic nature, *M. maripaludis* took about 1 week to grow in the control because the medium contains only 0.10% ammonium chloride.

EXPERIMENT 2

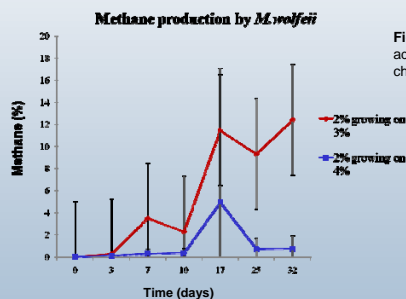


Figure 3: The 2% *M. wolfeii* was able to adapt to the 3% and 4% ammonium chloride concentrations.

Methane production by *M. maripaludis*

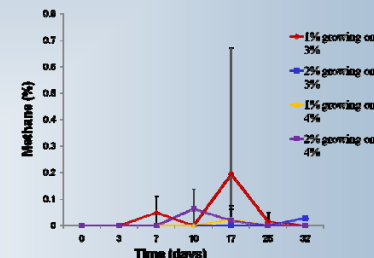


Figure 4: *M. maripaludis* was not very successful in growing at higher concentration of ammonium chloride.

CONCLUSIONS

- From the experiments, *M. wolfeii* grew at 4% ammonium chloride, while *M. maripaludis* grew on 3% ammonium chloride.
- A third experiment was conducted in which the 4% *M. wolfeii* was inoculated into 5 and 6% ammonium chloride. At this time, they have shown no methane production.
- Since *M. maripaludis* did not grow at higher concentrations of ammonium chloride, the hypothesis in that particular regard can be rejected.

BIBLIOGRAPHY

- [1] <http://saturn.nasa.gov/science/moons/enceladus/>
- [2] Christopher P. McKay et. al. (2008) *Astrobiology VIII*, 909-919.
- [3] Ricardo Cavicchioli. (2007) *Archae: Molecular and Cellular Biology*, 289.

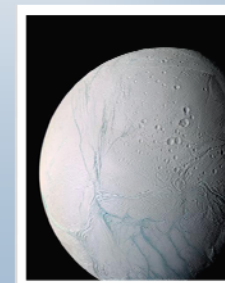


Figure 5: A composite picture of Enceladus taken by Cassini.